

# Depletion of Penicillin G Residues in Heavy Sows after Intramuscular Injection. Part I: Tissue Residue Depletion

S. J. Lupton,<sup>†</sup> W. L. Shelver,<sup>†</sup> D. J. Newman,<sup>‡</sup> S. Larsen,<sup>§</sup> and D. J. Smith<sup>\*,†</sup>

<sup>†</sup>Agricultural Research Service, Biosciences Research Laboratory, United States Department of Agriculture, 1605 Albrecht Boulevard, Fargo, North Dakota 58102-2765, United States

<sup>‡</sup>Department of Animal Science, North Dakota State University, 1300 Albrecht Boulevard, Fargo, North Dakota 58108, United States

<sup>§</sup>National Pork Board, 1776 NW 114th Street, Des Moines, Iowa 50325, United States

## S Supporting Information

**ABSTRACT:** Heavy sows ( $n = 126$ ) were treated with penicillin G procaine at a 5 $\times$  label dose (33 000 IU/kg) for 3 consecutive days by intramuscular (IM) injection using three patterns of drug administration. Treatments differed by injection pattern and injection volume. Sets of sows were slaughtered 5, 10, 15, 20, 25, 32, and 39 days after the last treatment; skeletal muscle, kidney, serum, and urine were collected for penicillin G analysis by LC–MS/MS. Penicillin G at withdrawal day 5 averaged  $23.5 \pm 10.5$  and  $3762 \pm 1932$  ng/g in muscle and kidney, respectively. After 15 days of withdrawal, muscle penicillin G residues were quantifiable in only one treated hog (3.4 ng/g) but averaged  $119 \pm 199$  ng/g in kidneys. Using a hypothetical tolerance of 50 ng/g and a natural log–linear depletion model, the withdrawal period required for penicillin depletion to 50 ng/g was 11 days for skeletal muscle and 47 days for kidney.

**KEYWORDS:** kidney, muscle, extra-label, penicillin G procaine, residue, sow, withdrawal period

## INTRODUCTION

Penicillin in its various forms has been used by the swine industry for decades but has not been typically associated with violative drug residues in animal carcasses destined for market. In 2012, over 3 million heavy sows were slaughtered in the United States,<sup>1</sup> mostly for use in the multibillion dollar<sup>2</sup> sausage industry.<sup>3</sup> Detections of penicillin residues in heavy sow tissues by the Food Safety and Inspection Service (FSIS) have increased substantially, resulting in carcass condemnations, after FSIS implemented a beta-lactam specific test.<sup>4,5</sup> Potential causes of such condemnation in addition to method changes could be due to extra-label usage without proper observation of withdrawal period, improper recommendation of withdrawal period, different depletion kinetics among the swine producing classes, or lack of a proper animal tracking system during the farm to packer marketing process. Because the United States has no tolerance for penicillin G residues in swine, any detectable penicillin G residue in an edible tissue would be considered violative.

Swine are typically treated with penicillin G procaine under extra-label conditions.<sup>6</sup> Under the Animal Medicinal Drug Use Clarification Act<sup>7</sup> (AMDUCA), producers, with the advice of a veterinarian, can legally use medicines on an extra-label basis, provided a preslaughter withdrawal time sufficient to clear violative residues from edible tissues is observed. Data for depletion of penicillin residues from swine tissues are scant, particularly in penicillin G procaine salt form used under extra-label usage conditions. Moats et al.<sup>8</sup> treated market hogs (80–110 kg) at 13 200 IU/kg (2 $\times$  label dose) and slaughtered the animals at 4 h, and at 1, 2, 4, and 8 days post treatment, and concluded that penicillin concentrations in muscle were quite variable but did not recommend a preslaughter withdrawal

period. A study conducted with 2 $\times$  and 10 $\times$  label doses in market pigs (96–104 kg) by Korsrud et al.<sup>9</sup> with withdrawal periods of 1, 2, 3, 5, and 7 days provided a foundation for Health Canada to recommend an 8-day withdrawal period for swine treated with a 2 $\times$  label dose and a 15 day preslaughter withdrawal period for animals treated with a 10 $\times$  label dose.<sup>9</sup> Apley et al.<sup>10</sup> conducted a depletion trial using heavy sows (~200 kg) dosed with a 5 $\times$  penicillin G procaine label dose with withdrawal periods of 2, 4, 6, and 8 days. A 28-day withdrawal period was recommended for kidney (the regulatory target tissue) even though penicillin detection after an 8 day withdrawal period was 60% in kidney. The three studies all have relatively short time periods relative to the last dose for the final sampling time. As described by Apley et al.,<sup>10</sup> an optimal residue depletion study, especially for estimating withdrawal periods, would result in all animals testing negative for the presence of penicillin residues in target tissues at the final sampling time.

Penicillin G procaine elimination rate in swine could be affected by variables including production class, dose, administration route, and pattern. Because of the apparent increased violation rate of penicillin G in heavy sows coincident with the regulatory method change, a study using three administration patterns that employed a typical extra-label dose was conducted. Treated sows were then harvested after a series of withdrawal periods extending to 39 days. The FSIS analytical method for  $\beta$ -lactam antibiotics<sup>11</sup> along with that of Apley et

Received: March 28, 2014

Revised: June 30, 2014

Accepted: July 8, 2014

Published: July 8, 2014

al.<sup>10</sup> were adapted to determine penicillin G concentrations at injection sites, serum, urine, kidney, and skeletal muscle. Liver and adipose tissue residues were not measured because neither is a regulatory target tissue for detecting penicillin G residues. Depletion kinetics of penicillin G subsequently were modeled to determine preslaughter withdrawal period recommendations for kidney and muscle.

## MATERIALS AND METHODS

**Study Overview.** To determine the effect of intramuscular (IM) administration pattern on penicillin G depletion, 126 heavy sows were administered a 5× label dose of penicillin G procaine for 3 consecutive days. The three penicillin G treatments differed in injection volume and pattern. Animals were serially slaughtered with 5, 10, 15, 20, 25, 32, and 39 day withdrawal periods, and urine, serum, kidney, skeletal muscle, and injection site were collected for penicillin G analyses by liquid chromatography–mass spectrometry (LC–MS/MS). Because of the large number of animals, the study was conducted in two identical trials of 63 animals each.

**Animals.** Prior to the initiation of the study, an animal use protocol was approved by the North Dakota State University (NDSU) Animal Care and Use Committee. One-hundred sixty heavy sows, in lots of 80 each, were purchased from the North Dakota Pig Cooperative (Larimore, ND). Sows were group housed in concrete-floored pens covered with ample quantities of straw as bedding. Animals were ear tagged and group-fed approximately 2 kg of a corn–soybean ration (Table 1) per animal at approximately 0800 h each day; pens were

**Table 1. Sow Diets Fed on a Restricted Basis (2 kg/animal per day)**

ingredient	kg	percentage
corn, ground	723.6	79.6
soybean meal (46.0%)	123.2	13.5
malt sprouts	45.5	5.0
monocal, 21%	0.9	0.1
limestone	7.8	0.9
salt	3.6	0.4
RalCo sow	4.5	0.5
	909.1	100.0

cleaned daily, and water was available on an ad libitum basis. At delivery, sows were sorted into groups that were lame, had visible abscesses, or other visible anomalies. These animals were separated from the other sows and were not considered for inclusion in the study. Within the pool of healthy animals, sows were randomly allocated to treatment and pen so that each pen contained 21 sows. At this time, individual sows were randomly allocated to withdrawal period.

Approximately 7 days prior to the initiation of penicillin G treatment, sows were tattooed according to their treatment assignment. An apparatus allowed multiple patterns of 2 cm “O”s to be tattooed onto necks of sows according to treatment (Figure 1). Tattooing allowed a consistent adherence to treatment protocols and designations for dose delivery.

**Treatments.** All sows were dosed with penicillin G procaine (Norocillin; Norbrook Laboratories, Lenexa, KS) at 33 000 U/kg BW. Treatments (illustrated in Figure 1) were designed to mimic the extra-label administration of penicillin G procaine thought to be used by veterinarians and swine producers during the treatment of infected sows.<sup>6</sup> Thus, each treatment differed in the volume and pattern of penicillin G procaine administration:

- Treatment 1: “10 mL; Single Site”. A maximum injection volume of 10 mL using multiple injection sites within day to deliver the total required drug volume; across day, injections were administered into the same site as the previous day (Figure 1, top panel).

- Treatment 2: “10 mL; Multiple Sites”. A maximum injection volume of 10 mL using multiple injection sites within day to deliver the total required drug volume; across day, injection sites were separated by approximately 5 cm (Figure 1, middle panel).
- Treatment 3: “20 mL; Multiple Sites”. A maximum injection volume of 20 mL using multiple injection sites within day to deliver the total required drug volume; across day, injections were separated by approximately 5 cm (Figure 1, lower panel).

The day prior to penicillin G procaine administration, sows were weighed. At dosing, penicillin G Procaine was administered via intramuscular injection through 3.8 cm, 16-gauge needles. Dosing was uneventful.

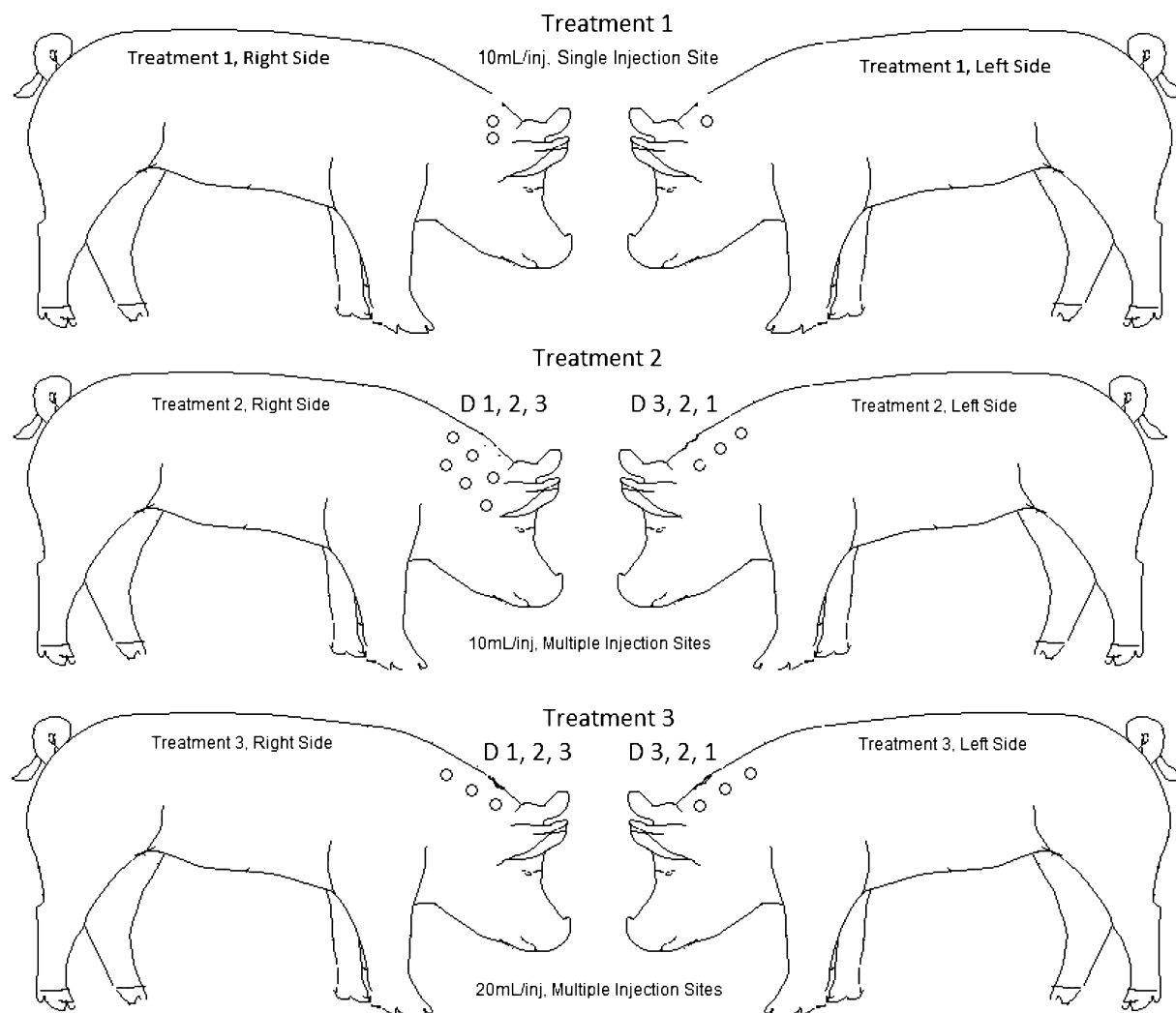
In addition to treatment animals, two positive control hogs received the label dose of penicillin G procaine by IM (6600 U/kg BW) injection for 3 consecutive days; during both trials, a single positive control sow was euthanized after a 7-day withdrawal period, consistent with the product label instructions. The other positive control sows were scheduled to be slaughtered after a 15-day withdrawal period; however, in trial 1, the 15 withdrawal day positive control sow died 12 days after penicillin G administration due to a ruptured spleen, so both 15-day withdrawal positive controls were obtained during trial 2. For both trials, untreated control sows were administered saline via intramuscular administration at 1 mL per 45.5 kg BW for 3 consecutive days and slaughtered after 5 days.

**Slaughter and Tissue Sampling.** Sows were euthanized according to American Veterinary Medicine Association recommendations<sup>12</sup> after 5, 10, 15, 20, 25, 32, or 39 day withdrawal periods relative to the last dosing day. Approximately 15 h prior to slaughter, sows designated for slaughter were withheld from access to feed. Sows were stunned by electrocution and captive bolt, after which they were quickly exsanguinated. Viscera were removed, and kidney and skeletal muscle (mid portion of the *longissimus dorsi*) were collected as well as urine and blood samples. At collection, samples were placed on dry ice; they were subsequently transported to the Biosciences Research Laboratory and stored at −80 °C until processing and analysis.

**Tissue Processing.** Frozen tissue samples were homogenized with dry ice using a Waring 1 L stainless steel blender (Torrington, CT) at a sample to dry ice ratio of approximately 1 to 1. Homogenized samples were subsequently placed at −20 °C to allow overnight sublimation; after sublimation of CO<sub>2</sub>, samples were moved to a −80 °C freezer until analysis.

**Extraction.** Apley et al.’s<sup>10</sup> analytical method was adapted to accommodate larger volumes for serum and urine analyses. One milliliter of serum or urine and 4 mL of HPLC-grade acetonitrile were mixed for 15 min. Samples were then centrifuged for 20 min at 3000g (4 °C); supernatants were transferred to clean labeled tubes and were evaporated under N<sub>2</sub> at 45 °C to a volume of 0.5 mL. Concentrated extracts were quantitatively transferred to 1.5 mL microcentrifuge tubes, diluted to the 1 mL mark with HPLC-grade water, fortified with deuterated penicillin G standard (d<sub>7</sub>, 100 ng; Sigma-Aldrich), and vortexed for 15 s. Extracts were then centrifuged at 17 000g for 20 min and aliquoted into glass vials for same-day LC–MS/MS analysis. Matrix-matched standard curves were prepared with each sample set having a concentration range of 2–500 pg/μL. Samples having penicillin G concentrations greater than the highest calibration standard were diluted with water into the quantitative range of the calibration curve as were contemporaneously run matrix-matched calibration standards. The extraction efficiency for serum was 88.7 ± 8% (*n* = 16) with method limits of detection (mLOD) and quantification (mLOQ) of 1.5 and 5.0 pg/μL, respectively. The urine extraction efficiency was 98.1 ± 8.0% (*n* = 16) with mLOD and mLOQ of 1.1 and 4.1 pg/μL, respectively.

Kidney, muscle, and injection site samples were extracted following the FSIS method CLG-BLAC.02 for β-lactam screening and confirmation<sup>11</sup> with minimal modifications. To kidney samples (2 g) was added 10 mL of an 80/20 mixture of HPLC-grade acetonitrile/nanopure water, and tubes were shaken for 15 min and centrifuged at 3000g for 15 min (4 °C). Sample extracts were then added to a



**Figure 1.** Penicillin G procaine treatments. Sows were administered 5X doses (33 000 IU/kg BW) of penicillin G procaine on each of three consecutive days as either 10 mL injections (treatments 1 and 2) or 20 mL injections (treatment 3). Across day, treatment 1 sows received injections in a single location; sows in treatments 2 and 3 received consecutive injections at locations separated by approximately 5 cm intervals. Because sows were very large, total injection volumes were typically greater than 20 mL; thus, for treatments 1 and 2, the pattern of injections was 10 mL right, 10 mL left, and the remaining volume (for total doses greater than 20 mL) would be injected into the right side approximately 5 cm below the initial 10 mL injection. The injection pattern for treatment 3 was 20 mL in the right side with overflow injections occurring in the left neck muscles. Injection sites were marked by tattoos 7 days prior to the initial treatment.

prepared (5 mL of acetonitrile and 2 × 5 mL of nanopure water) Bakerbond Octadecyl (C18, 1000 mg; JT Baker) solid-phase extraction (SPE) cartridge. Samples were allowed to flow through SPE cartridges by gravity; SPE cartridges were dried under vacuum. Unretained eluents were subsequently evaporated to 1 mL under N<sub>2</sub>, syringe filtered through a PTFE 0.45 μm filter, and 100 ng of deuterated penicillin G was added. Extracts were centrifuge-filtered through a Millipore Amicon Ultra centrifuge filter (3000 nominal molecular weight limit) at 14 500g for 30 min, and 100 μL was placed into an autoinjector vial for LC–MS/MS analysis. The extraction efficiency for kidney was 72.7 ± 10.7% (*n* = 26) with mLOD and mLOQ of 1.8 and 6.1 ng/g, respectively.

Muscle and injection site samples were extracted with 10 mL of an 80/20 mixture of HPLC-grade acetonitrile/water saturated with hexane. Samples were then shaken for 15 min and centrifuged at 3000g for 15 min at 4 °C. The supernatants were decanted into a clean 50 mL tube, 5 mL of hexane was added, and extracted for 5 min with shaking. Samples were then centrifuged at 3000g for 15 min at 4 °C, the hexane layer was removed, and the extracts were processed as described for kidney by SPE and filtration. The extraction efficiency for

muscle and injection site was 55.8 ± 9.4% with mLOD and mLOQ of 0.7 and 2.4 ng/g, respectively.

Duplicate blank (negative control) and fortified samples (25 ng/g penicillin G procaine) were extracted with each sample set. Trial samples were extracted in duplicate by withdrawal day. Before analysis, a penicillin G-d7 internal standard was added to each sample extract at an end concentration of 100 pg/μL. Blank sample extracts were utilized to prepare matrix matched standard curves ranging from 2 to 500 pg/μL. A new standard curve was made fresh for each sample set to avoid any penicillin G degradation. Samples and curves were analyzed by UPLC–MS/MS within 24 h of extraction. Samples with penicillin G concentrations above the highest standard in the curve were re-extracted and diluted with water before analysis. Blank matrices for standard curves of diluted samples were diluted with water to ensure equivalent matrix-matching.

**Ultra High Performance Liquid Chromatography–Mass Spectrometry.** A modified version of the UHPLC–MS/MS method described by Apley et al.<sup>10</sup> was used to determine penicillin G concentrations. Extracts were analyzed using a Waters (Milford, MA) ultra high performance liquid chromatograph coupled to a tandem quadrupole mass spectrometer (Acquity). The chromatography was modified slightly to be a slower gradient to affect a longer retention of

**Table 2.** Depletion of Penicillin G Residues (mean  $\pm$  std dev) from Skeletal Muscle, Kidney, Serum, and Injection Site of Heavy Sows<sup>a</sup>

WD period (d)	treatment			<i>P</i> <sup>b</sup>	overall average (ng/g or ng/mL)
	1 (ng/g or ng/mL)	2 (ng/g or ng/mL)	3 (ng/g or ng/mL)		
Skeletal Muscle <sup>c</sup>					
5	20.0 ± 6.3	20.1 ± 10.5	30.4 ± 11.9	0.14†	23.5 ± 10.5
10	4.8 ± 5.9 <sub>xy</sub>	1.3 ± 2.1 <sub>x</sub>	9.8 ± 6.4 <sub>y</sub>	0.004†	5.3 ± 6.1
15	0.8 ± 0.7	1.0 ± 1.2	0.7 ± 0.6	0.81‡	0.8 ± 0.8
20	1.1 ± 1.0	1.0 ± 1.4	0.6 ± 0.4	NC	0.9 ± 1.0
25	0.4	0.4	0.4	NC	0.4
32	0.4	0.6 ± 0.3	0.4	NC	0.5 ± 0.2
39	0.4	0.4	0.4	NC	0.4
Kidney <sup>d</sup>					
5	3751 ± 676	3238 ± 1110	4298 ± 3219	0.81†	3762 ± 1932
10	782 ± 1034 <sub>xy</sub>	100 ± 210 <sub>x</sub>	1119 ± 919 <sub>y</sub>	0.03†	667 ± 875
15	183 ± 315	42.1 ± 65.5	133 ± 138	0.61‡	119 ± 199
20	65.4 ± 140	22.0 ± 51.7	46.2 ± 111	0.42‡	44.5 ± 103
25	25.6 ± 60.4	15.7 ± 23.1	0.9	0.36‡	14.1 ± 36.6
32	16.1 ± 26.0	28.5 ± 67.6	0.9	NC	15.2 ± 41.0
39	0.9	4.5 ± 8.9	3.6 ± 6.6	NC	3.0 ± 6.2
Serum <sup>e</sup>					
5	466 ± 169	368 ± 120	486 ± 122	0.33†	440 ± 141
10	89.6 ± 116	9.7 ± 17.1	164 ± 170	0.02†	87.8 ± 129
15	12.0 ± 18.5	7.6 ± 11.2	15.2 ± 13.9	0.51‡	11.6 ± 14.3
20	5.3 ± 11.1	1.0 ± 0.6	6.7 ± 14.6	0.98‡	4.3 ± 14.6
25	3.0 ± 5.6	0.8	0.8	NC	1.5 ± 3.2
32	1.0 ± 0.7	2.0 ± 3.0	0.8	NC	1.2 ± 1.7
39	0.8	1.4 ± 1.5	0.8	NC	1.0 ± 0.8
Injection Site <sup>c</sup>					
5	2 910 000 ± 1 230 000	1 600 000 ± 1 680 000	3 570 000 ± 3 150 000	0.18†	2 690 000 ± 2 220 000
10	428 000 ± 686 000	787 ± 1860	500 000 ± 430 000	0.01‡	310 000 ± 494 000
15	118 000 ± 286 000	80.0 ± 121	493 ± 581	0.63†	39 400 ± 165 000
20	2350 ± 5720	30 700 ± 75 100	16.5 ± 24.0	0.52†	11 000 ± 43 300
25	98 300 ± 241 000	4120 ± 10 100	2.9 ± 5.8	0.54‡	34 200 ± 139 000
32	20 600 ± 50 500	0.4	0.4	NC	6880 ± 29 200
39	3.5 ± 7.6	1.0 ± 1.3	212 ± 511	NC	72.2 ± 295

<sup>a</sup>Data are expressed as ng of penicillin G per g of tissue. Individual values below each matrix limit of quantitation (LOQ), but above the method limit of detection (LOD), were included in means as nominal values returned by the LC–MS/MS assay. Values below the matrix LOD were included in mean calculations at 1/2 of the LOD. Mean values shown in italic font were below the method limit of quantification (LOQ) but above the method limit of detection (LOD); mean values shown in bold font were below the method LOD and are expressed at one-half the LOD. Means within a row with differing letters (*x*, *y*) differ ( $P < 0.05$ ). Means within withdrawal period and treatment were calculated from six animals each. Data from individual animals are included in the Supporting Information. <sup>b</sup>Within withdrawal period means were ln transformed and compared using one-way ANOVA using parametric (<sup>†</sup>) or nonparametric (<sup>‡</sup>) tests; NC, not compared. <sup>c</sup>Skeletal muscle and injection site data are not corrected for recovery, which averaged 55.8  $\pm$  9.4% across all skeletal muscle assays ( $n = 14$ ); method LOQ, 2.4 ng/g LOD, 0.7 ng/g; one-half LOD, 0.4 ng/g. <sup>d</sup>Kidney data are not corrected for recovery, which averaged 72.7  $\pm$  10% across all kidney assays ( $n = 26$ ); method LOQ, 6.1 ng/g; LOD, 1.8 ng/g; one-half LOD, 0.9 ng/g. <sup>e</sup>Serum data are not corrected for recovery, which averaged 88.7  $\pm$  9.4% across all serum assays ( $n = 14$ ); method LOQ, 5.0 ng/g; LOD, 1.5 ng/g; one-half LOD, 0.8 ng/g.

penicillin G relative to the Apley et al.<sup>10</sup> method. Penicillin G was separated from matrix on a Waters BEH C18 column (2.1  $\times$  50 mm, 1.8  $\mu$ m) using a mobile phase gradient consisting of: 0 to 1.5 min, isocratic 80/20 10 mM ammonium acetate in water/10 mM ammonium acetate in acetonitrile (A/B); 1.5 to 1.75 min, linear gradient to 20/80 A/B; 1.75 to 4 min, isocratic 20/80 A/B; flow rate of 0.8 mL/min. Penicillin G and deuterated penicillin G eluted at 0.45 min. Analytes were ionized in positive electrospray mode using a capillary voltage of 3.5 kV at a desolvation temperature of 500 °C and a nitrogen flow rate of 800 L/h. Other parameters included a source temperature of 150 °C and a cone voltage of 35 V.

The detection method was also modified to monitor additional fragment ions for penicillin G to improve confirmation and quantification of residues. Fragment ion isolation and detection was accomplished in the multiple reaction monitoring mode. For penicillin G and deuterated penicillin G, two fragments were monitored. The

precursor ion for penicillin G was 335 *m/z*; using a collision energy of 10, fragment ions at 160 *m/z* and 176 *m/z* were produced. For deuterated penicillin G, the precursor ion was 342 *m/z*; with a collision energy of 12, fragment ions at 160 *m/z* and 183 *m/z* were produced. If sample duplicate concentrations differed by more than 25%, they were re-extracted and reanalyzed in duplicate to achieve better agreement between replicates. Reported data are not corrected for recovery.<sup>13</sup> Method LODs were calculated as the standard deviation of seven replicates of fortified samples (2 ng/mL or ng/g) multiplied by the Student's *t* value (99% confidence) with  $n - 1$  degrees of freedom; mLOQs are 10 times the standard deviation of the seven replicates.<sup>14</sup>

**Statistical Comparisons.** Within withdrawal period and matrix, data were natural log (ln) transformed prior to analyses by simple one-way ANOVA. For time points in which assumptions of normality (tested using the Shapiro–Wilk method) were met, differences in



Table 3. Depletion of Penicillin G Residues (mean  $\pm$  std dev) from Urine of Heavy Sows<sup>a</sup>

WD period (d)	treatment			<i>p</i> <sup>b</sup>	overall average (ng/mL)
	1 (ng/mL)	2 (ng/mL)	3 (ng/mL)		
5	338 000 $\pm$ 473 000	378 000 $\pm$ 252 000	282 000 $\pm$ 234 000	0.62‡	332 000 $\pm$ 320 000
10	41 100 $\pm$ 57 600 x,y	6490 $\pm$ 10 200 x	78 600 $\pm$ 70 400 y	0.04†	42 100 $\pm$ 58 200
15	11 000 $\pm$ 20 700	9960 $\pm$ 17 000	26 400 $\pm$ 27 000	0.35‡	15 800 $\pm$ 22 000
20	2870 $\pm$ 4580	2220 $\pm$ 5440	4410 $\pm$ 10 800	0.87‡	3170 $\pm$ 7080
25	2800 $\pm$ 6840	487 $\pm$ 1040	0.8 $\pm$ 0.6	NC	1090 $\pm$ 3950
32	2270 $\pm$ 3770	8030 $\pm$ 19 700	0.6	NC	3430 $\pm$ 11 400
39	0.6	585 $\pm$ 1430	880 $\pm$ 2150	NC	489 $\pm$ 1450

<sup>a</sup>Data are expressed as ng of penicillin G per mL of urine. Individual values below each matrix limit of quantitation (LOQ; 4.1 ng/mL), but above the method limit of detection (LOD; 1.1 ng/mL), were included in means as nominal values returned by the LC–MS/MS assay. Values below the matrix LOD were included in mean calculations at 1/2 of the LOD. Means within a row with differing letters (x, y) differ ( $P < 0.05$ ). Means within withdrawal period and treatment were calculated from six animals each. Data from individual animals are included in the Supporting Information.

<sup>b</sup>Within withdrawal period means were ln transformed and compared using one-way ANOVA using parametric (†) or nonparametric (‡) tests; NC, not compared.

treatment means were determined using standard parametric methods (i.e., Holm–Sidak). In instances in which normality was not met, nonparametric analysis was conducted using the Kruskal–Wallis one-way ANOVA on ranks. Specific details on the use parametric or nonparametric tests for any tissue/withdrawal period combination are shown within each data table.

**Estimation of Withdrawal Period.** Withdrawal periods were estimated using the method described by the US FDA-CVM<sup>13</sup> with some modification. In the estimation of withdrawal periods for kidney, the following criteria were used. These criteria are a combination of European Union and US FDA approaches to handling data from residue depletion trials. First, for tissue residue data for a given withdrawal day to be included in the analysis, at least three animals had to have returned residues above the method limit of quantification.<sup>13</sup> Second, nominal values below the limit of quantification, but above the limit of detection, were used as returned by the assay if there were at least three points at the withdrawal period above the limit of quantification. Third, values falling below the limit of detection were included at one-half the method limit of detection.<sup>15</sup>

For kidney, data obtained from withdrawal days 10, 15, 20, and 25 were used for the calculation of withdrawal period. Data from day 32 were not employed even though there were three animals with penicillin residues above the LOQ because the 32-day data were not linear ( $P = 0.45$ ) with respect to withdrawal day 20 and 25 data. It should be noted, however, that the 10, 15, 20, and 25 day data set violated the statistical assumptions of normality and equal variance.

To estimate the withdrawal period for skeletal muscle, only one animal out of 18 had residues above the LOQ at the 15-d withdrawal period, with three animals having residues above the LOD, but below the LOQ. Because a minimum of three time points are required to determine the terminal, linear elimination period, the 15-d withdrawal period data were used to estimate a withdrawal period for skeletal muscle. In doing so, the day 15 data included one value that was above the LOQ, four animals that had residue values between the LOQ and LOD, and 13 animals in which no detectable residue was present; for the latter animals, a value of one-half the LOD was used for calculations. Skeletal muscle data also violated the statistical assumptions of normality and equal variance.

Estimations of withdrawal period were completed for kidney and skeletal muscle tissues using the procedure described by the US-FDA CVM in guideline no. 3, “General Principles for Evaluating the Safety of Compounds used in Food Producing Animals”.<sup>13</sup> Excel 7.0 was used in conjunction with the tables of Owen<sup>16</sup> to calculate the critical values of the noncentral *t*-distribution used in FDA calculations.

## RESULTS AND DISCUSSION

Weights of sows dosed with penicillin G procaine averaged 234.2, 223.8, and 226.8 kg for treatments 1, 2, and 3, respectively ( $\pm 4.5$  kg; pooled SEM) and did not differ ( $P =$

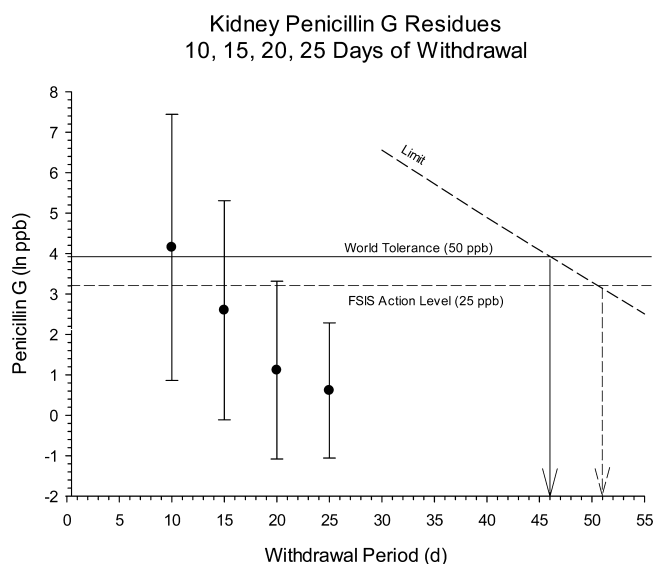
0.24). Average slaughter weights were 237.4, 226.5, and 228.9 kg ( $\pm 4.4$  kg; pooled SEM) and did not differ ( $P = 0.19$ ).

Mean residues of penicillin G in skeletal muscle, kidney, serum, and injection site are shown in Table 2. Table 3 shows mean urinary depletion of penicillin G by treatment and withdrawal period. Raw (individual animal) data are presented as Supporting Information.

The depletion of penicillin G residues from kidneys of heavy sows is presented in Figure 2. The pattern of penicillin G administration had no discernible effect on residue depletion, so treatments were pooled. Quantifiable residues were present in kidneys of all 18 animals 5 days after the final penicillin G procaine injection, but by 10 days 6 of 18 (33%) animals had residues below the assay LOD (1.8 ng/g). By 20 days of withdrawal, 5 of 18 animals had residues above the LOQ with three of these animals having kidney residues above 100 ng/g and one animal with penicillin G residues greater than 300 ng/g. Penicillin G residues were present in the kidneys of two hogs after a 39-d withdrawal period. The presence of penicillin G residues in 11% of animals (2 of 18 animals) at withdrawal day 39 suggests that an extensive withdrawal period would be required for the complete depletion of penicillin from kidneys of a population of treated animals.

Regulatory organizations in the European Union and the United States estimate preslaughter withdrawal periods using nearly identical techniques.<sup>13,15</sup> That is, withdrawal periods are calculated by determining the time required for tissue residues in 99% (U.S.) or 95% (Europe) of a population of animals to deplete to an established tolerance with 95% confidence. Tolerance limits are calculated using linear regression of the natural logs (ln) of tissue residues in the linear depletion phase of the residue depletion curve. In calculating the 95th percentile confidence interval, the U.S. assumes a noncentral *t*-distribution,<sup>13</sup> whereas the European Union allows the 95th percentile confidence interval to be calculated using the standardized normal distribution.<sup>15</sup> The two methods generally return similar results,<sup>15,17</sup> depending upon a number of variables, the most important being the handling of data which fall below the method limit of quantification.<sup>17</sup> Both the U.S. and the CVMP methods of establishing withdrawal periods have been criticized<sup>18,19</sup> because of the difficulty with which residue data collected over time meet assumptions of normality and equal variance.

A withdrawal period was estimated for kidney tissues using the ln-linear approach promulgated by the US-FDA CVM<sup>13</sup>



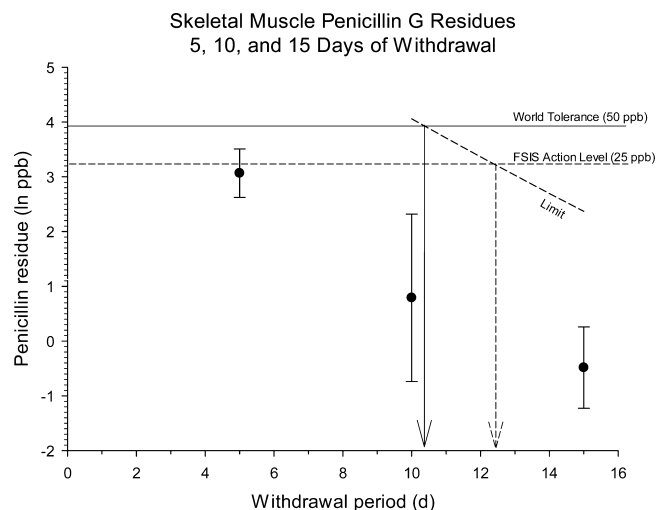
**Figure 2.** Estimation of preslaughter withdrawal period in heavy sows treated with a 5 $\times$  dose of penicillin G procaine, based on depletion of penicillin G residues from kidney tissues. Data taken from the linear portion of the depletion curve were analyzed as described by FDA Guidance Document no. 3 (US FDA CVM, 2006). In contrast to US FDA guidance, 15-d withdrawal data below the method LOD were assigned values of 1/2 the LOD (CMVP, 1995). Kidney penicillin G concentrations that were between the LOQ and LOD were included in the analysis as their nominal concentration. The residue tolerance used by most of the world (50 ng/g or ln 3.91 ng/g) crossed the 99th percentile with a 95th percent confidence interval tolerance limit at approximately 46.5 days (solid vertical arrow). The kidney withdrawal period for a tolerance of 50 ng/g was established at 47 days after rounding up to the nearest whole-day. In the United States, the tolerance level is zero for penicillin G in swine tissues, but FSIS has established an action limit of 25 ng/g for penicillin residues detected in swine tissues (FSIS, 2013). For penicillin residues to deplete below the action limit of 25 ng/g in a population of swine, just over 50 days would be required; rounding up to the nearest whole-day, the withdrawal period would be 51 days.

with modifications suggested by the CVMP.<sup>15</sup> In making a withdrawal period estimation, the essential assumptions of equal variance and normal distributions (Shapiro–Wilk) of data were violated ( $P < 0.001$ ). That is, because the withdrawal period estimate is based on the assumption that the variance of the upper 1% of a population is known, the withdrawal period parameter is sensitive to assumptions associated with the variance model.<sup>20</sup> As discussed by Concordet and Toutain<sup>18</sup> and Riviere,<sup>20</sup> and as documented by Sanquer et al.<sup>19</sup> and Chiesa et al.,<sup>21</sup> assumptions of equal variances are difficult, and sometimes impossible to meet. Nonparametric approaches to withdrawal period calculations proffered by Sanquer et al.<sup>19</sup> and Concordet and Toutain<sup>19</sup> were not attempted on this data set.

Poor confidence in the variance estimates governing the withdrawal parameter notwithstanding, Figure 2 shows that an estimated 47-day withdrawal period would be required for kidney residues to deplete to 50 ng/g in 99% of the animals (with 95% certainty) in a population of heavy sows treated with a 5 $\times$  dose of penicillin G procaine for 3 consecutive days. For residues to fall below the FSIS action level of 25 ng/g<sup>22</sup> in a population of treated sows, an estimated 51-day withdrawal period would be required. Again, these estimates are not statistically valid because the data from which they were calculated were not normally distributed and did not have an

equal variance. Nevertheless, the empirical evidence itself suggests that had a statistically sound data set been generated, an actual withdrawal period based on kidney residues would be prohibitively long with regards to commercial sow production systems in the United States.

In contrast to kidney tissues, penicillin G residues depleted quickly from skeletal muscle (Table 2, Figure 3). Residues in



**Figure 3.** Estimation of preslaughter withdrawal period in heavy sows treated with a 5 $\times$  dose of penicillin G procaine, based on depletion of penicillin G residues from skeletal muscle. Data taken from the linear portion of the depletion curve were analyzed as described by FDA Guidance Document no. 3 (US FDA CVM, 2006). In contrast to US FDA guidance, withdrawal data below the method LOD were assigned values of 1/2 the LOD (CMVP, 1995). Skeletal muscle penicillin G concentrations that were between the LOQ and LOD were included in the analysis as their nominal concentrations. The residue tolerance used by most of the world (50 ng/g or ln 3.91 ng/g) crossed the 99th percentile with a 95th percent confidence interval tolerance limit at approximately 10.4 days (solid vertical arrow). The skeletal muscle withdrawal period for a tolerance of 50 ng/g was estimated to be 11 days after rounding up to the nearest whole-day. In the United States, the tolerance level is zero for penicillin G in swine tissues, but FSIS has established an action limit of 25 ng/g for penicillin residues detected in swine tissues (FSIS, 2013). For penicillin residues to deplete below the action limit of 25 ng/g in a population of swine, an estimated 13 days would be required.

skeletal muscle at 5 days of withdrawal averaged only  $23.5 \pm 10.5$  ng/g and depleted rapidly thereafter. By the 15th day of withdrawal, only 1 sow of 18 dosed had skeletal muscle residues greater than the method LOQ (2.4 ng/g), with 4 other sows having residues greater than the method LOD (0.7 ng/g), but less than the method LOQ. Thus, the estimated withdrawal periods for skeletal muscle were 11 days (Figure 3) for the worldwide tolerance of 50 ng/g, and 13 days for the FSIS action level of 25 ng/g. Regardless of dosing pattern, the Food Animal Residue Avoidance Database-estimated withdrawal time of 15 days proposed by Payne et al.<sup>23</sup> in 2006 (and at the time this study was conducted) was sufficient for penicillin residues to deplete from skeletal muscle. Again, it should be noted that an 11-day withdrawal period does not represent a regulatory withdrawal period because the normality assumption inherent in regression analyses was not met.

Table 2 contains residue depletion data from day three (last exposure) injection sites collected at slaughter. Across all treatments, residues in injection sites collected 5 days after the

last administration of penicillin G were very high, averaging over 2.6 million ng/g of tissue; however, the data also indicate that injection site residues were highly variable. At withdrawal day 15 and later, the relative variation in residue concentration was approximately 400% of the mean. Such variation at injection sites has been described previously<sup>19</sup> and is typical of injection site residue data. Because of this variability, no long-term-effect of injection pattern was discerned from the data. Kosrud et al.<sup>9</sup> suggest that the proportion of an intramuscular dose that is delivered intramuscularly will influence the kinetics of penicillin depletion. In practice, however, ensuring uniform, intramuscular delivery of injectable penicillin G is difficult in heavy sows, especially when injected into the neck.

From a broad perspective, the actual method that a producer uses to deliver extra-label doses of penicillin G to a sow is unknown by regulators. Therefore, pooling injection site data, or residue data from other tissues, across treatments may provide the best indication of how penicillin residues deplete in the larger swine population, which, no doubt, is subject to a variety of injection techniques. The pooled data ("overall average" column of Table 2) clearly indicate that injection site residues are greater than skeletal muscle residue. Supporting Information tables showing residues from individual animals, however, indicate that high average residues usually occurred through the contributions of only a few animals per withdrawal period.

For penicillin, the target tissue for regulatory monitoring is kidney.<sup>24</sup> While kidneys are not commonly consumed in the U.S., it is possible that muscle from injection sites could be consumed.<sup>25</sup> Injection sites are not typically defined as a target tissue because they are difficult to locate on an animal carcass, injection sites containing drug residues are applicable to only a small number of market animals, and injection sites are not a reliable tissue for monitoring drug exposure in a population of animals.<sup>26</sup> In addition, residues from injection sites deplete at inconsistent rates and are highly variable, which makes calculating withdrawal periods based on injection site residues difficult.<sup>19</sup> Finally, the probability of a consumer actually being exposed to an injection site in a food product is extremely low. Because tolerances are based on acceptable daily intakes of a given residue over assumed lifetime exposures,<sup>25,27</sup> and because tolerances are established with several inherent safety factors, the probability of an adverse toxic effect occurring, even in the event of exposure to residue at an injection site, is extremely low.

Actual probability of risk notwithstanding, food animal producers would generally distance themselves from marketing products containing residues that may be perceived as risky. For penicillin, overt toxicity to the general population is not a concern because penicillin is of low toxicity;<sup>28</sup> in individuals with allergic hypersensitivity to penicillin, however, residue might initiate an allergic reaction. For example, use of penicillin in dairy cows and delivery of penicillin residues in milk to sensitive individuals was a problem in the decade after the introduction of penicillin to veterinary use.<sup>29–31</sup> The degree of allergic reaction to penicillin, ranging from mild to severe, depends upon the sensitivity of presensitized individuals, the route of penicillin exposure, and the dose of penicillin residue received.<sup>30,32</sup> Estimates of the doses of penicillin required to initiate allergic response range from 3 to 6  $\mu\text{g}$ <sup>28,32</sup> with doses required to sensitize individuals being much greater. While the probability of delivering sufficient penicillin to sensitized individuals is very low, examples of allergic reactions in

intentionally<sup>33</sup> and unintentionally<sup>34</sup> penicillin-exposed (via meat) individuals have been documented. For unintentional exposures, documented reactions are exceedingly rare.<sup>28,35</sup>

For swine producers, the presence of penicillin residues in food products could be mitigated by several practices. First, producers using extra-label penicillin could observe the estimated withdrawal periods of 50 days or greater in all treated sows, which would allow residues in edible tissues to deplete. However, observation of a 7-week withdrawal period would not be economical in sows destined for market.

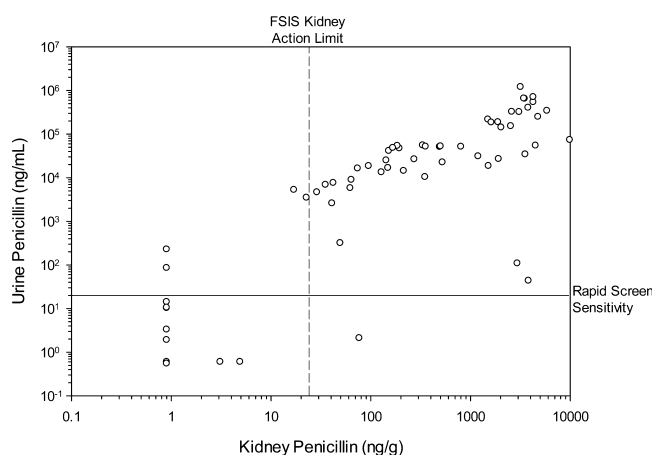
A second option would be to discontinue penicillin use altogether. Such action, however, is not likely justifiable based on combined animal welfare and human food safety considerations. As pointed out by the European Food Safety Authority,<sup>36</sup>  $\beta$ -lactam antibiotics (including penicillin) "are among the most important group of antimicrobial agents in veterinary medicine", and they have been used in animal agriculture for decades. For example, the first U.S. approval for penicillin occurred in 1951,<sup>37</sup> and currently approximately 99.5% of all penicillin use in swine is administered for disease prophylaxis and treatment.<sup>38</sup> It is telling that with regards to food safety, the main criticism of penicillin use resides not with toxicity concerns or even allergic responses to residues, but to the hypothesis that  $\beta$ -lactam use in food animals could contribute to the transmission of antimicrobial resistance.<sup>37,38</sup> Thus, the elimination of penicillin use in food animals is not likely justified on the basis of food safety.

A third option would be to market penicillin-treated animals with a withdrawal period sufficient for muscle residues to deplete, and to discard the kidneys with inedible offal for rendering. While kidney is the target tissue for penicillin in swine carcasses,<sup>24</sup> residues in kidney do not represent a risk if they are not available for human consumption. In the United States, only a small percentage of the value of a market sow is associated with kidney; therefore, discard of kidney for rendering would not likely represent a hardship for processing plants that handle heavy sows. Admittedly, such discard might not be economically viable in markets in which sow kidney has a greater relative value.

As attractive as kidney discard would appear to be in the United States for solving penicillin residue issues, regulatory agencies will not likely allow such action because kidney is also the target tissue for a number of other drugs. For example, in the United States, the Food Safety and Inspection Service's screening and confirmatory method for a multitude of residues is based on kidney.<sup>22</sup> Even with the retention of renal tissue at slaughter, producers do have the option, suggested by Payne et al.,<sup>23</sup> of screening animals to ensure that they are penicillin-free prior to marketing. Table 3 shows that urinary penicillin residues are substantially more concentrated than kidney residues (Table 2) at any given slaughter time. Thus, the presence of penicillin in urine is an excellent predictor that the antibiotic has not completely depleted from tissues, whereas there is little evidence (Table 2) that serum would serve in such capacity. Using instrumental analysis such as LC–MS/MS to perform urinary penicillin residues analysis, however, would be difficult to provide timely feedbacks to producers to ensure that the animals are penicillin G free. Using urine and kidney samples from the current study, Shelver et al.<sup>39</sup> demonstrated that the kidney inhibition swab test (KIS test, Charm Sciences, Inc.; Lawrence, MA) rapid screening assay, employed by the FSIS to screen kidney tissues at slaughter facilities, when used with urine samples very accurately predicted Charm-KIS



positive kidney samples and was an excellent predictor of residues greater than the 25 ng/g action limit in use by the US FSIS.<sup>22</sup> Figure 4 demonstrates the within-animal relationship



**Figure 4.** Within-animal relationship between urinary penicillin G residues (Y-axis) and renal penicillin G concentrations across all slaughter times. The horizontal solid line represents the limits of detection of the Charm-KIS rapid screening assay in urine,<sup>39</sup> and the vertical hatched line shows the FSIS “minimal level of applicability” for penicillin G residues in swine kidney (FSIS-2013). Points below the solid line would not be detected by a urinary rapid screening assay; points to the left of the vertical hatched line would not be flagged by the FSIS. Use of the urinary assay would be conservative due to the fact that the screen would have flagged four animals as “positive” when the kidney results would have been flagged as negative. A single sample would have passed the urine screen while kidney residues remained above the FSIS action level.

between renal and urinary penicillin concentrations. With one exception, urinary penicillin concentrations were excellent predictors of potentially violative kidney residues ( $n = 121$ ). Such data suggest that the kidney inhibition swab test, when used with a urine matrix, is a viable option for a timely (<5 h run time) on-farm screen for the presence of penicillin residues.

Data presented in this study clearly demonstrate that penicillin residues deplete at disparate rates in edible tissues in swine treated with extra-label doses. From a human food safety perspective, the earlier FARAD 15-day withdrawal period that is commonly observed for extra-label penicillin doses<sup>23</sup> is sufficient for residue depletion from skeletal muscle. It would take approximately 50 days for residue to deplete to nondetectable levels in kidneys from a population of sows treated with an extra-label penicillin dose, concurring with the current FARAD recommendation (which is based on these data). This study demonstrates the disparity between residue depletion in tissues sometimes selected for regulation of drug residues (kidney) and tissues for which the majority of the value of a carcass resides (skeletal muscle). This disparity could leave valuable animal-health drugs vulnerable to discontinuation, not due to human food safety concerns, but due to regulatory inflexibility.

## ■ ASSOCIATED CONTENT

### Supporting Information

Results on an individual animal basis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Tel.: (701) 239-1238. E-mail: [david.j.smith@ars.usda.gov](mailto:david.j.smith@ars.usda.gov).

### Funding

This study was collaboratively funded by the National Pork Board (58-5442-2-404), the USDA ARS (project no. 5442-32000-014), North Dakota State University, and in-kind contributions provided by Charm Sciences (agreement no. 58-5442-32000-014-07n).

### Notes

The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable. USDA is an equal opportunity employer.

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

The technical expertise of Roberta Dahlen, Dee Ellig, Austen Germolous, Justin Gilbertson, Nathaniel Grosz, Kelsey Heiberg, Grant Herges, Dillon Hoffsommer, Jason Holthusen, Benjamin Klinkner, Erin Loeb, Amy McGarvey, Richelle Miller, Colleen Pfaff, and Terry Skunberg is greatly appreciated as are the efforts of student employees of the North Dakota State University Meats Laboratory and Animal Physiology and Nutrition Center.

## ■ REFERENCES

- (1) USDA Agricultural Marketing Service. 2012 Annual Meat Trade Review. <http://www.ams.usda.gov/mnreports/l sancmtr.pdf>, accessed 6/25/2014.
- (2) Salvage, B. Most US sausage categories are growing, thanks to product diversity and bold business moves. MeatPoultry.com, January 8, 2014. <http://www.meatpoultry.com/Writers/Bryan%20Salvage/Sausage%20tsunami.aspx>, accessed 6/25/2014.
- (3) Meyer, S. R. Sausage market fundamentals taking hold. National Hog Farmer, September 8, 2009. <http://nationalhogfarmer.com/weekly-preview/0908-sausage-market-fundamentals>, accessed 6/25/2014.
- (4) JAVMA. Tissue tests locate penicillin G residues, JAVMA news, October 1, 2011. <https://www.avma.org/News/JAVMANews/Pages/111001j.aspx>, accessed 1/8/2014.
- (5) National Hog Farmer. Warning Issued for Penicillin Residues in Sows. National Hog Farmer, July 21, 2011. <http://nationalhogfarmer.com/health-diseases/warning-issued-penicillin-residues-sows>, accessed 1/8/2014.
- (6) Hoover, T. C. Health and performance improvements of pigs treated with DRAXXIN (tulathromycin) injectable solution for swine respiratory disease under field conditions. March 31, 2009. The Pig Site. <http://www.thepigsite.com/articles/1/pig-health/2674/health-and-performance-of-pigs-treated-with-draxxin>, accessed 1/8/2014.
- (7) Code of Federal Regulations. Twenty-one CFR Part 530.
- (8) Moats, W. A.; Harris, E. W.; Steele, N. C. Depletion of intramuscularly injected procaine penicillin G from tissue of swine. A comparison of HPLC and bioassay procedures. *J. Agric. Food Chem.* **1986**, *34*, 452–456.
- (9) Kosrud, G. O.; Salisbury, C. D. C.; Rhodes, C. S.; Papich, M. G.; Yates, W. D. G.; Bulmer, W. S.; MacNeil, J. D.; Landry, D. A.; Lambert, G.; Yong, M. S.; Ritters, L. Depletion of penicillin G residues in tissues, plasma and injection sites of market pigs injected intramuscularly with procaine penicillin G. *Food Addit. Contam.* **1998**, *15*, 421–426.



- (10) Apley, M.; Coetzee, H.; Gehring, R.; Karriker, L. Pharmacokinetics and tissue residues of procaine penicillin G in sows after administration of 33,000 IU/kg intramuscularly and by needle-free injection in the hip. National Pork Board Research Report NPB #07-234, 2009.
- (11) USDA FSIS. 2011. Screening and Confirmation of  $\beta$ -Lactam Antibiotics by HPLC-MS/MS, CLG-BLAC.02. Effective 07/02/2011.
- (12) AVMA. AVAMA Guidelines for the Euthanasia of Animals, 2013 ed.; American Veterinary Medical Association: Schaumburg, IL, 2013.
- (13) US FDA CVM. 2006. Guidance for Industry, #3. General principles for evaluating the safety of compounds used in food-producing animals. U.S. Dept. Health and Human Services. <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052180.pdf>.
- (14) Harris, D. C. *Quantitative Chemical Analysis*, 8th ed.; W. H. Freeman and Co.: New York, NY, 2010.
- (15) CVMP. Note for guidance: Approach towards harmonisation of withdrawal periods. European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products, 1995.
- (16) Owen, D. B. *Handbook of Statistical Tables*; Addison-Wesley Publishing Co., Inc.: Reading, MA, 1962.
- (17) Damte, D.; Jeong, H.-J.; Lee, S.-J.; Cho, B.-H.; Kim, J.-C.; Park, S.-C. Evaluation of linear regression statistical approaches for withdrawal time estimation of veterinary drugs. *Food Chem. Toxicol.* **2012**, *50*, 773–778.
- (18) Concordet, D.; Toutain, P. L. The withdrawal time estimation of veterinary drugs revisited. *J. Vet. Pharmacol. Ther.* **1997**, *20*, 380–386.
- (19) Sanquer, A.; Wackowicz, G.; Havrileck, B. Critical review on the withdrawal period calculation for injection site residues. *J. Vet. Pharmacol. Ther.* **2006**, *29*, 355–364.
- (20) Riviere, J. *Comparative Pharmacokinetics. Principles, Techniques, and Applications*, 2nd ed.; Wiley-Blackwell: Ames, IA, 2011.
- (21) Chiesa, O. A.; Li, H.; Kijak, P. J.; Li, J. X.; Lancaster, V.; Smith, M. L.; Heller, D. N.; Thomas, M. H.; von Bredow, J. Tissue /fluid correlation study for the depletion of sulfadimethoxine in bovine kidney, liver, plasma, urine, and oral fluid. *J. Vet. Pharmacol. Ther.* **2011**, *35*, 249–258.
- (22) US FSIS. Screening and confirmation of animal drug residues by UHPLC-MS-MS. CLG-MRM1.02, 2013.
- (23) Payne, M. A.; Craigmill, A.; Riviere, J. E.; Webb, A. I. Extralabel use of penicillin in food animals. *J. Am. Vet. Med. Assoc.* **2006**, *229*, 1401–1403.
- (24) NADA 065-010. Freedom of Information Summary: Norocillin, Penicillin G procaine injectable suspension cattle, sheep, swine, and horses. <http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM218419.pdf>.
- (25) Reeves, P. T. Residues of veterinary drugs at injection sites. *J. Vet. Pharmacol. Ther.* **2007**, *30*, 1–17.
- (26) CVMP. Revised reflection paper on injection site residues: Considerations for risk assessment and residue surveillance. European Medicines Agency, Committee for Medicinal Products of Veterinary Use. ([http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2013/10/WC500152656.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/10/WC500152656.pdf)), 2013.
- (27) Oriani, J. US Food and Drug Administration's perspective on aquaculture drug residues. In *Xenobiotics in Fish*; Smith, D. J., Gingerich, W. H., Beconi-Barker, M. G., Eds.; Kluwer Academic/Plenum Publishers: New York, 1999.
- (28) CVMP. Penicillins, Summary Report. European Medicines Agency, Veterinary Medicines and Inspection, Committee for Veterinary Medicinal Products. [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Maximum\\_Residue\\_Limits\\_-\\_Report/2009/11/WC500015568.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015568.pdf), 2008.
- (29) Ormerod, A.; Reid, T.; Main, R. A. Penicillin in milk: its importance in urticaria. *Clin. Exp. Allergy* **1987**, *17*, 229–234.
- (30) Siegel, B. B. Hidden contacts with penicillin. *Bull. W. H. O.* **1959**, *21*, 703–713.
- (31) Wicher, K.; Reisman, R. E.; Arbesman, C. E. Allergic reaction to penicillin in milk. *JAMA, J. Am. Med. Assoc.* **1969**, *208*, 143–145.
- (32) Dewdney, J. M.; Maes, L.; Raynaud, J. P.; Blanc, F.; Scheid, J. P.; Jackson, T.; Lens, S.; Verschueren, C. Risk assessment of antibiotic residues of  $\beta$ -lactams and macrolides in food products with regard to their immuno-allergic potential. *Food Chem. Toxicol.* **1991**, *29*, 477–483.
- (33) Lindemayr, H.; Knobler, R.; Kraft, D.; Baumgartner, W. Challenge of penicillin-allergic volunteers with penicillin-contaminated meat. *Allergy* **1981**, *36*, 471–478.
- (34) Raison-Peyron, N.; Messaad, D.; Bousquet, J.; Demoly, P. Anaphylaxis to beef in penicillin-allergic patient. *Allergy* **2001**, *56*, 796–797.
- (35) Dayan, A. D. Allergy to antimicrobial residues in food: assessment of risk to man. *Vet. Microbiol.* **1993**, *35*, 213–226.
- (36) European Food Safety Authority. Scientific opinion on the public health risks of bacterial strains producing extended  $\beta$ -lactamases and/or AmpC  $\beta$ -lactamases in food and food-producing animals. *EFSA J.* **2011**, *9*, 2322.
- (37) Institute of Medicine. Human health risks with the subtherapeutic use of penicillin or tetracyclines in animal feed. *Report by the Committee of Human Health Risk Assessment of Using Subtherapeutic Antibiotics in Animal Feeds*, Institute of Medicine, IOM-88-89; National Academy Press: Washington, DC, 1989.
- (38) Cox, L. A.; Popken, D. A.; Mathers, J. J. Human health risk assessment of penicillin/aminopenicillin resistance in enterococci due to penicillin use in food animals. *Risk Anal.* **2009**, *29*, 796–805.
- (39) Shelver, W. L.; Lupton, S.; Newman, D. J.; Larsen, S.; Smith, D. J. Depletion of penicillin G residues in heavy sows after intramuscular injection Part II: Application of kidney inhibition swab tests. *J. Agric. Food Chem.* **2014**, DOI: 10.1021/jf501494w.